DEVELOPMENT AND COMPARISON OF SPECTROGRAPHIC METHODS FOR DETERMINATION OF ZINC IN PLANT MATERIAL; SURVEY OF THE ZINC CONTENT OF KANSAS GROWN ALFALFA

by

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B. S., Kansas State College
of Agriculture and Applied Science, 1947

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Chemistry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1948

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INTRODUCTION

There are known to exist in the earth's crust, over 90 elements. The literature shows that sixty of these elements have been positively identified in plants (33). The majority of these are present, however, only in minute quantities. These elements are known as minor elements or trace elements.

The minor elements have been divided roughly into two classes, the essential and nonessential elements. There is, at the present time, considerable disagreement as to which elements should be placed in each division, but the following are generally recognized as essential: zinc, iron, boron, manganese, and copper. As the result of extended study, the effect of this group of elements on plant growth is reasonably well established. The elements such as lead, titanium, barium, and strontium fall into the nonessential group. Their effect on plant growth remains, as yet, to be determined.

A thorough study of the functions of minor elements in plant growth and metabolism is comparatively recent. Previous to 1932, the work with zinc consisted of more or less emperical experiments. With the development of accurate methods of analysis for micro quantities, the interset pertaining to minor elements in plant metabolism has increased greatly. Indicative of this interest is the element zinc. Literature shows over 250 papers dealing with zinc deficiencies in the United States alone (4). This number is mounting rapidly as methods of analysis for zinc become more thoroughly established.

Knowledge that zinc is essential in plant growth is not new. Earliest accounts indicate that zinc was first postulated to be essential for the growth of a fungus, *Aspergillus niger*, in 1869 (7). Javillier (19) through his work with yeast, found increased activity and consumption of sugar solution by the yeast in the presence of zinc. Maze (23, 24) working at the same time, determined, by water culture methods, that zinc was necessary for the growth of corn, though his work, like that of Javillier, was not well accepted. Not until the work of Sommer and Lyman (38) and Sommer (39) was zinc widely accepted as an essential plant nutrient.

Zinc deficiencies are a world-wide occurrence. The United States and 20 South and Central American countries, Europe, and the Far East have reported zinc deficiencies (8). Among the plants most commonly affected are citrus (41), pineapples (22), pecans (1), deciduous fruits (42), and corn (3). Zinc, however, has also been shown to be essential in the growth of oats (26), wheat (27), barley (39), soybeans (10), green beans (15), and others. There remain many crops which have not had their zinc requirements established.

The symptoms of zinc deficiency usually involve some form of chlorosis. Citrus, deciduous, and other trees deficient in zinc characteristically show mottled leaf, little leaf or rosette, or bronzing. These symptoms are generally associated with some form of chlorosis. The symptoms of zinc deficiency may be summarized as follows: some form of leaf chlorosis, reduced leaf size, malformation, scant foliage, and reduced fruit production

(8).

Zinc salts first came into commercial use as a fertilizer in 1932. Since that time the use of these salts as plant nutrients has grown widely. Chlorosis or yellowing of the corn plant, usually called white bud, appears within a week or two after the seedlings emerge from the ground. This condition may be controlled and overcome by the addition of relatively small quantities of zinc sulfate placed in the rows before planting (3). Zinc sprays (2) and zinc dust (29) have been shown to be effective in controlling diseases of citrus trees.

The exact function of each minor element in the plant is not known singularly. The following are some of the postulated functions of zinc: metabolic activities, catalysis, change pH of soil through chemical reaction, determine oxidation states of other elements present, liberate other elements lower in electromotive series, build resistance in the plant to disease or parasites, and stimulate an effect comparable to the effect of vitamins on animals (45). Zinc deficiencies are accompanied by chlorosis, and lacking contrary evidence, it may be justifiably postulated that zinc is related to the formation of chlorophyll (8).

There are no reported zinc deficiencies in Kansas, and it is believed that there have been no surveys of any Kansas grown crop to determine its zinc content. For this reason it seemed advisable to make such a survey. Alfalfa was chosen as the crop on which to make this survey because of the increasingly important role it is playing in the development of the state. With increased dehydration of the crop, dehydrated alfalfa has become an important source of protein and vitamins and is used as feed for livestock and poultry. Since the boron, sodium, iron, copper, manganese, potassium, phosphorus, and calcium content of Kansas grown alfalfa is already known, it was felt that a survey of the zinc content of the same samples would represent a valuable addition to the data already available. The samples used for the zinc survey were the same as those used for the boron survey previously made by Smith, Schrenk, and King (37).

SURVEY OF THE LITERATURE

The analysis for zinc in the minute quantities in which it occurs in plant material is at best a very difficult one. Any analysis to be satisfactory must be sensitive to very small amounts of zinc, and in addition must be sensitive to small concentration changes. Several methods of analysis have been proposed, but those gaining the most favor are colorimetric and spectrographic.

Hibbard (17) devised a micro method for the determination of zinc using hydrogen sulfide in a solution of carefully controlled pH. This method was subject to interference by other elements precipitated by the hydrogen sulfide. Vanselow and Laurence (43) described a spectrographic procedure using the zinc line at 3345.0 Angstroms, with a preliminary concentration of the zinc by hydrogen sulfide. Cadmium, which was used as an internal standard, was precipitated with the zinc. Research has shown that preliminary concentration of the zinc is necessary if the line at 3345.0 Angstroms is to be used. Scott and Mitchell

(35) described a similar spectrographic technique, but used 8-hydroxyquinoline (oxime) as a means of concentrating the zinc.

Rogers (31) recommended the use of the zinc line at 2138.5 Angstroms which does not require a preliminary chemical concentration. Specially sensitized plates are necessary if this line is to be used. Rogers and Gall (32), compared the use of the 2138.5 Angstrom line without concentration, and with chemical methods of concentration followed by spectrographic analysis using the 3345.0 Angstrom line. They gave an unfavorable report on the latter procedure. They also reported that the 2138.5 Angstrom line is 30 to 50 times as sensitive as the 3345.0 Angstrom line. O'Conner (28) has reported on a modified procedure similar to that of Rogers, which he has applied successfully to mixed fertilizers.

Increasing consideration has been given to adsorptiometric determination of zinc, the most promising results being obtained from the use of diphenylthiocarbazone (dithizone) as a color developing agent. Sandell (34) has employed a colorimetric dithizone method based on the work of Fischer and Leopoldi (11) for the determination of zinc in soils. The dithizone gives a red to purple color when added to a solution of zinc, although copper, lead and cobalt present in the solution will interfere. The color developed has an absorption maximum at 525 mμ. It is possible through control of pH to separate out the interfering elements as copper, lead and cobalt (18). Cowling and Miller (9) have suggested a "mixed color" reaction which involves the use of sodium diethyldithiocarbamate (carbamate) in addition to dithizone to act as an inhibitor to the metals previously mentioned. This modification, however, has the disadvantage that the carbamate limits the complete extraction of the zinc. This objection may be overcome by careful control of pH, volume of carbamate added, and volume of the aqueous phase. This method has been adopted as a tentative method of analysis for zinc by the Association of Official Agricultural Chemists (9).

Reed and Cummings (30) have described a polarographic determination of zinc in the presence of all the constituents ordinarily present in plant ash except those removed from the ash extract at a pH between four and five. Merrit (25) has suggested the use of 8-hydroxyquinolinate as a reagent to develop fluorescence with small quantities of zinc. Benzoin has also been suggested by White and Neustadt (44) as a qualitative fluorescent reagent for zinc.

Work in this laboratory has shown the hydrogen sulfide concentration of zinc suggested by Vanselow and Laurance (43) to be inconsistent and tedious. Since Rogers and Gall (32) have found this method unfavorable too, it seemed advisable to investigate the concentration procedure of Scott and Mitchell (35). This investigation has led to a modification of the spectrographic technique of Scott and Mitchell (35) which gives results with good accuracy, precision, and values which compare favorably with those obtained with the dithizone colorimetric technique.

EXPERIMENTAL PROCEDURE

Description of Apparatus

The spectrograph used in this research was a Bausch and Lomb large Littrow instrument with interchangeable quartz and glass optics. The quartz optics were used for this research. This instrument has a wave length range of 2100 to 8000 Angstrom units and a linear dispersion of 1.5 Angstrom units per millimeter at a wave length of 2150 Angstrom units and six Angstrom units per millimeter at a wave length of 3350 Angstrom units. The quartz prism is of the Littrow type, 57 millimeters high with a reflection coating of evaporated aluminum. The combination collimating and focusing lens has a focal length of 1827 millimeters and is provided with two stops for reducing aperture.

The illuminating system consisted of the electrode holders, a spherical condensing lens, and a rotary sector disk. Line densities were obtained by means of an Allied Research Laboratories-Dietert densitometer.

The samples were excited with a direct current generator capable of producing a potential of 150 volts, the current depending on the resistance of the circuit. A ballast resistor was placed in series with the generator to stabilize the current.

Preliminary Work

The zinc spectrum has two lines which are considered especially suitable for quantitative analysis. They are located at 2138.6 Angstroms and 3345.0 Angstroms. The first of these two lines is sufficiently sensitive to permit analysis of the plant ash without chemical treatment. The second is, however, less sensitive and the zinc occurring in plant material must be concentrated by some chemical means previous to spectrographic analysis. Table 1 shows a list of the possible interfering lines and their wave lengths (6).

Table 1. Possible interfering lines that appear in the region of the zinc lines located at 2138.6 and 3345.0 Angstroms.

Element	Line Angstroms	Relative line intensity
Zinc	2138.56	800R
Copper	2138.5	25
Iron	2138.589	8
Zinc	3345.0	800
Chromium	3345.2	15
Molybdenum	3344.7	50
Cerium	3344.8	50

Rogers (31) and O'Connor (28) have shown that the copper and iron lines do not interfere if they are present in concentrations not exceeding one and three percent respectively. Although chromium, molybdenum, and cerium are listed as having possible interfering lines in the region of the zinc line at 3345.0 Angstroms, no difficulty is experienced for two reasons: first, the interfering elements are not present in sufficient quantity to give visible spectral lines in this region; and second, the dispersion of the instrument in this region is sufficient that they would be resolved.

Calibration Curve for Position Five

Since the spectrum length of the large Littrow spectrograph is about 80 centimeters, it is possible to photograph only a limited portion of it at a time. There are 10 different positions of the plate and prism necessary to photograph the entire spectrum length. Position 10 was selected to photograph the zinc line at 2138.6 Angstroms and position five for the zinc line at 3345.0 Angstroms.

Because the instrument may not be in the same adjustment as when it left the factory, and the focus and tilt prescribed by the manufacturer may not be the best for the region of the spectrum in which the zinc line occurs, it was thought advisable to check the recommended values of focus and tilt. This was done by photographing a series of iron spectra with the tilt set at the recommended value and varying the focus a few divisions on each side of the given value. Examination of the plate after development showed the focus to be best at 108.5 for position five. Making a similar plate setting the focus at 108.5 and varying the tilt, the tilt was found to be best at 241. These settings were used for all analyses in position five. Similar plates were made in position 10 and the best focus and tilt were found to be 218 and 136, respectively.

Because the dispersion of a prism is not linear, it was necessary to prepare a calibration curve for each position. To do this a millimeter scale was printed across the top of the plate and directly below this an iron spectrum. After development of the plate, the wave lengths of several iron lines were determined by comparison with a standard iron spectrum provided with the instrument by the manufacturer. After location of a few wave lengths in this manner, others were determined using the iron spectrum charts compiled by Brode (6). The wave lengths thus determined were plotted against the scale readings to obtain the dispersion curve, Fig. 1.

Selection of Internal Standard

Gerlach and Schweitzer (14) were the first to report the use of an internal standard to compensate for variations likely to occur in the arc during the excitation of the sample. The internal standard may be one of the following two types: a weak line of a major constituent of the sample; of a small,

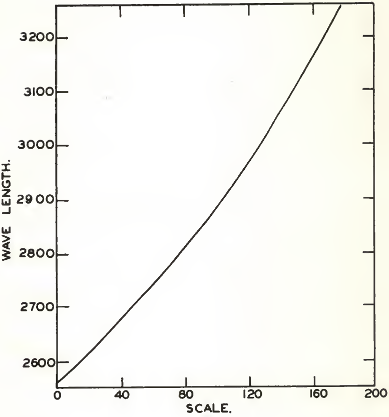

FIG.I. DISPERSION CURVE.

unvarying amount of an element added to each sample. Preparation of a series of standard samples with varying amounts of zinc and a constant amount of a reference element, followed by observation of the intensity ratio between the reference element and the zinc lines, provides the basis of a working curve.

The internal standard method assumes that variations in excitation conditions will effect the unknown and reference element in the same manner. Also, the difference in densities of the two lines is assumed to be proportional to the logarithm of the ratio of the intensities of these lines in the source. The latter is true only for the straight line portion of the characteristic curve of the photographic emulsion in question.

In making a series of exposures for the same sample the line densities vary widely. This is especially true of an element such as zinc which is highly volatile. The principal reason for this lies in the fact that it is impossible to control the many variations in the arc which will tend to change the rate of vaporization of the sample. The probability of exciting the vaporized atom will then change with time. In order to account for these variations the internal standard should have the following characteristics:

1. The internal standard should have an excitation voltage similar to that of the analysis element.

2. The internal standard and analysis element should have nearly the same rate of vaporization; their boiling points

should be similar.

3. The internal standard line should be in the same spectral region as the line of the element sought.

4. The internal standard should be present in such a concentration that its line density is approximately the mean value of the density of the element sought in this concentration range.

5. The internal standard should be homogeneously mixed with the sample.

There have been a number of lines suggested for use as an internal standard for the determination of zinc. Hess, Owens, and Reinhardt (16) in their analysis of organic materials, used molybdenum and bismuth. Rogers (31) suggested the use of tellurium as an internal standard in the analysis of plant ash. O'Connor (28) in the analysis of mixed fertilizers suggested the use of beryllium as internal standard. Vanselow and Laurence (43) in the analysis of plant ash used cadmium as an internal standard. Cadmium was chosen for use as the internal standard in this analysis of alfalfa for zinc.

It was desirable to select an internal standard element which could be used with both the zinc line at 2138.6 Angstroms and at 3345.0 Angstroms, and still possess as many of the previously mentioned characteristics as possible. In practice, no element fulfills all of them, however, in this case, cadmium meets the majority of the requirements for both lines. Both cadmium and zinc have similar excitation voltages, being 8.96 and 9.36 volts, respectively. The two elements have nearly the same boiling points, cadmium 767° C. and zinc 907° C. Cadmium has spectral lines near the 2138.6 and 3345.0 Angstrom zinc lines, located at 2144.4 and 3261.1 Angstrom units. The remaining two requirements are easily met in the establishment of experimental conditions. In the case of analysis using the zinc line at 2138.6 Angstroms, the internal standard is added in liquid form to each ash sample; and when the line at 3345.0 Angstroms is used, thoroughly mixed liquid samples are employed.

SPECTROGRAPHIC ANALYSIS OF ALFALFA USING THE ZINC LINE AT 2138.6 ANGSTROMS

Preparation of the Standards

Since extraneous elements are known to effect relative line intensities (20), the standards should have as nearly as possible the same composition as the samples. When using the zinc line at 2138.6 Angstroms it is possible to use the plant ash directly, and it was found necessary to prepare a synthetic ash mixture similar in composition to the plant ash, but completely free of zinc. This synthetic ash mixture, or base powder, is composed of 5.0 g of potassium dihydrogen phosphate, 16.7 g of potassium carbonate, 19.6 g of calcium carbonate, and 3.28 g of magnesium oxide. These chemicals were combined, thoroughly mixed, and placed in a muffle at 450 to 475° C. overnight to convert all compounds to the oxides. The resulting dry powder was similar in composition to the plant ash.

A solution of 0.33 percent $CdCl_2 \cdot 2\frac{1}{2} H_2O$ in hydrochloric acid (1:1) was used as the internal standard. The standard zinc solution contained one mg of zinc per milliliter. A series of standards were made by adding varying amounts of the standard zinc solution with water, and a constant amount of the internal standard, as shown in Table 2.

Table 2. Dilutions used for making a series of spectrographic standards for use with the zinc line at 2138.6 Å.

Standard number	Int. Std. solution ml	Zinc soln. ml	Water ml	Amt. zinc on electrode ppm
1	5	0.2	4.8	2
2	5	0.5	4.5	5
3	5	1.0	4.0	10
4	5	2.0	3.0	20
5	5	3.0	2.0	30

In order to form the working curve shown in Fig. 2, an appropriate amount of the base powder was weighed on to each electrode and 0.1 ml of the spectrographic standard was added. The electrodes were then dried in an air oven at 100° C. for several hours. All chemicals used in these analyses were checked spectrographically to show that zinc was not present as an impurity.

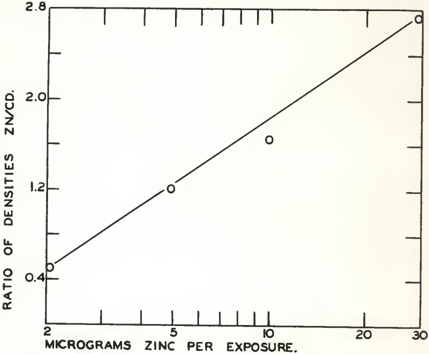

FIG.2. WORKING CURVE FOR ZINC 2138 LINE.

Determination of Exposure

In working with mixed fertilizers, O'Connor (28) used a logarithmic sector which reduced exposure by 75 percent and found a two and one half minute exposure to be satisfactory. It was necessary to verify this exposure under the modified conditions which were employed. This was accomplished by making a moving plate spectrum of the standard containing the highest concentration of zinc. Examination of the plate showed the sample to be satisfactorily volatilized at the end of two minutes.

It was found that this exposure time produced excessive background on the photographic plate. To correct this condition, the rotating sector was used. This permitted the spectrum of the sample to be taken during the complete excitation period, however, it reduced the total exposure by an amount proportional to the sector opening. The intensity of the zinc line was decreased appreciably when the sector was more than 1/8 closed. If the number of interruptions for each exposure exceeds 50, Brode (6) has reported that the intermittency effect is negligible. In the analyses reported here the number of interruptions greatly exceeds the minimum, 50.

Preparation of Electrodes

Spectrographic graphite electrodes purchased from the

National Carbon Company were used in these analyses. They were prepared for use by cutting the 5/16 inch carbons into 7/8 inch lengths. The anode was prepared by drilling a well in one end of the electrode, nine to ten mm in depth with walls one to one and one half mm thick. The cathode was shaped with a sharp point similar to a pencil.

The anode was prepared for the sample by filling the well with a solution of carbon tetrachloride saturated with carnauba wax. Following this the electrodes were dried in an air oven for 15 minutes at a temperature of 100° C.

Spectrographic Procedure

Two g of dry plant material were ashed in a porcelain crucible at a temperature of 450 to 475° C. The percent ash was noted and 30 mg samples of the ash weighed into each electrode well. Following this, 0.1 ml of internal standard solution, containing a concentration of cadmium equivalent to that in the spectrographic standards, was placed on the samples and the electrodes were dried in an air oven.

Eastman type 103-0 ultraviolet sensitive plates were selected as the most sensitive plates available. The samples were arced with a d.c. potential of 150 volts and a current of 12.5 amperes for a period of two minutes with the sector 7/8 open. The slit width was set at 50 mμ. A series of standards were run on each plate.

After exposure the plates were washed for one minute in ethylene chloride and allowed to dry before further development. Following this the plates were developed for three minutes at a temperature of 68° C. in Eastman D-19 developer, then fixed and dried. The line intensities were determined with the aid of an A.R.L.-Dietert densitometer. A slight background correction was necessary. The results were reported as parts per million on the basis of the dry plant material.

Since it is more convenient to work with solutions, experiments were conducted to determine the possibility of dissolving a known weight of ash in a hydrochloric acid solution. An effort was made to dissolve both 60 and 120 mg of plant ash in one milliliter of hydrochloric acid and an aliquot of the resulting solution was to be used for analysis. There was a large residue of insoluble material and the zinc lines obtained were too weak to be used for analysis and others were completely absent; therefore, these experiments were abandoned.

Precision of Analysis Using the Zinc Line at 2138.6 Angstroms

In order to select the better of two methods of analysis, it is essential to determine the precision and accuracy of each. The precision of this method was determined by several analyses on the same sample. Averages were obtained and the deviations and probable errors were calculated. Table 3 presents data which indicate the precision of the method in the analyses of a sample of commercially dehydrated alfalfa. The

Table 3. Analyses of commercially dehydrated alfalfa to show precision of the spectrographic method using the line at 2138.6 A°.

Cd	Zn	Zn/Cd	ppm	D	D^2
0.135	0.111	0.82	15.4	22.1	488.4
0.085	0.146	1.72	49.3	11.8	139.2
0.098	0.180	1.84	61.0	23.5	552.2
0.161	0.174	1.18	24.0	13.5	182.2
0.110	0.199	1.81	50.5	13.0	169.0
0.186	0.227	1.22	24.5	13.0	169.0
Total				224.7	1690.0
Average				37.5	

S = standard deviation = $\sqrt{\frac{\Sigma D^2}{n-1}} = \sqrt{\frac{1690}{5}} = 18.4$

Standard error = $S_x = \frac{S}{\sqrt{n}} = \frac{18.4}{2.24} = 8.21$

Probable error = $S_x \cdot \frac{\sqrt{2}}{2} = (8.21)(0.7) = 5.74$

Percent standard error = $\frac{8.21}{37.5}$ = 22 percent

Percent probable error = $\frac{5.74}{37.5}$ = 15.3 percent probable error was calculated to be 15.3 percent. This sample contained 35 parts per million of zinc when analyzed by the dithizone colorimetric technique (9) and 37.5 when analyzed spectrographically.

SPECTROGRAPHIC ANALYSIS OF ALFALFA USING THE ZINC LINE AT 3345.0 ANGSTROMS

Selection of the Spectrographic Buffer

Photoelectric densitometry has greatly increased the precision of measurement of spectral line intensities because small variations in these intensities can be detected. Several factors contribute to these variations which affect analytical data. Among these is the effect of extraneous elements in the arc. This condition is due at least in part to the transport mechanism of ions across the arc. Transport phenomena in arc sources may be the result of an equilibrium between thermal diffusion of material from the region of the electrode and a migration of ions of the sample due to electrical forces. Langstroth and McRae (20) have investigated transport phenomena and reached the following conclusions:

1. Lines of different elements have, in general, different intensity distributions along their lengths.

2. The relative distributions depend on ionization potentials and masses of the elements, and on the ionization potentials of other atoms present in the discharge.

3. When easily ionized atoms are present in the discharge, the distribution of an element relative to that of a more easily ionized element of comparable mass falls off more sharply toward the unloaded electrode than it does when easily ionized atoms are absent.

The purpose of the spectrographic buffer is to minimize variations in the transport mechanism from spectrum to spectrum. Requisites of an acceptable buffer are that it does not emit a complicated spectrum of its own, and that it has a low ionization potential.

Hess, Owens, and Reinhardt (16) have reported on a number of different buffers for use in the analysis of organic materials for trace impurities. They investigated the possibilities of lithium tartrate, potassium bromide with lithium tartrate, potassium nitrate with ammonium nitrate, and sodium nitrate. They found sodium nitrate the most satisfactory for stabilizing the arc conditions for the analysis of several metals. In the analysis of boron Smith (36) has reported favorably on the use of lithium chloride as a buffer.

Since the majority of spectrographic work in this laboratory is done using chloride salts, the use of lithium chloride as a buffer was investigated. Further work led to the selection of lithium chloride as a buffer because it seemed to possess several of the desired characteristics.

A typical calibration curve is shown in Fig. 3. The slight curve at the lower concentration occurs because the lowest point falls on the toe of the plate characteristic curve;

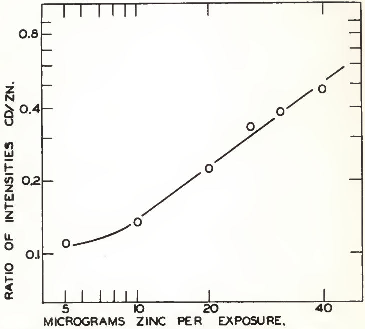

FIG. 3. WORKING CURVE FOR ZINC 3345 LINE.

the remainder is on the straight line portion of this curve.

The buffer for the data reported in this paper was prepared by making a five percent solution of reagent grade lithium chloride using hydrochloric acid (1:1) as the solvent. This solution was diluted with an equal volume of standard zinc solution and water to give a final buffer concentration of two and one half percent.

Preparation of Standards

In the preparation of standards the buffer solution was made up as previously described, but containing in addition 0.05 percent $CdCl_2 \cdot 2\frac{1}{2}\ H_2O$ as an internal standard. The zinc standard is the same as used previously; it contains one mg of zinc per ml. A series of standards was made by adding varying amounts of the standard zinc solution with water to a constant amount of buffer and internal standard, as shown in Table 4.

In order that the standards would have approximately the same composition as the sample of plant ash after concentration, 0.1434 g of $MgSO_4 \cdot 7\ H_2O$ and 1.765 g of $Fe\ SO_4 \cdot NH_4SO_4 \cdot 6H_2O$ were added to each 100 ml of internal standard.

Table 4. Dilutions used for making a series of spectrographic standards for use with the zinc line at 3345.0 Å.

Standard number	Buffer and Int. Std. ml	Zinc soln. ml	Water ml	Amt. zinc per exposure ppm
1	5	0.25	4.75	5
2	5	0.5	4.5	10
3	5	0.75	4.25	15
4	5	1.0	4.0	20
5	5	1.5	3.5	30
6	5	2.0	3.0	40
7	5	3.0	2.0	60

Determination of Exposure

In order that the correct amount of zinc be observed in the sample or standard, it is necessary that the sample be completely vaporized. The rate of vaporization will depend on the material on the electrodes and the temperature of the arc.

By making a moving plate spectrum of a sample of the spectrographic standard containing the highest amount of zinc, it was found that all of the zinc was volatilized in approximately 20 seconds. In order to account for variations in the arc the samples were excited for 30 seconds.

Since this zinc line falls very near the region of the cyanogen bands, it is difficult to reduce plate background without seriously affecting line intensities. It is, however, possible, by use of the rotating sector, to reduce background to such a value that no correction is required. The sector was set at 5/16 open and rotated at a speed greatly exceeding 50 interruptions per exposure for all exposures.

Preparation of Electrodes

Ordinary graphite spectrographic carbons without purification or pre-arcing were found satisfactory for this determination. These carbons were purchased from the National Carbon Company, and were prepared for use by cutting the 5/16 inch carbons into 7/8 inch lengths. The anodes were shaped to have a cone-like crater about one and one half mm in depth. The cathode was shaped similar to the round nose of a bullet. Smith (36) has shown that electrodes shaped in this manner gave good precision and the arc had less tendency to wander.

Before placing solution on the electrodes, the crater of the anode was filled with an excess of solution of carbon tetrachloride saturated with carnauba wax. They were then dried in an air oven for approximately 15 minutes. After cooling, the electrodes were ready to receive the sample solutions.

Concentration of the Zinc by Means of 8-Hydroxyquinoline

As has been previously stated, the zinc present in plant material must be chemically concentrated for spectrographic analysis using the line located at 3345.0 Angstroms. Fleck and Ward (12) have shown that 8-hydroxyquinoline will quantitatively precipitate zinc from solution between a pH of 4.58 and 13.4. Berg (5) has shown it possible to quantitatively separate zinc from other metals, such as magnesium, copper, and cadmium, but for concentration purposes this separation procedure is not necessary. Scott and Mitchell (35) have reported a concentration procedure applicable to plant material using this reagent. Their technique, with slight modification, was applied to the alfalfa samples previous to spectrographic analysis.

Four grams of dry plant material were ashed in a porcelain crucible at a temperature of 450 to 475° C. The percent ash was noted, and the ash quantitatively transferred to a small evaporating dish. The ash was moistened with a small amount of distilled water and 15 milliliters of hydrochloric acid (1:1) were added. The solutions were placed on a uniformly heated hot plate and allowed to evaporate to half volume. By this time all acid soluble zinc had been extracted and the solution was filtered through a Whatman No. 41 filter paper, and the residue thoroughly washed with boiling water. The resulting solution was diluted to 150 ml with distilled water. Hibbard (17) and others have reported that the insoluble ash contains zinc. The residue from several samples was examined spectrographically and no zinc could be detected; if any were present the concentration was below the sensitivity of the method. In a majority of the samples the insoluble residue was small.

Ten ml of five percent 8-hydroxyquinoline in acetic acid (2 N) were added, followed by ammonia (1:1) until the solution became an emerald green. This color is due to the formation of a complex of the reagent with iron present. At this point the pH should range between 1.8 and 1.9. Fifty ml of ammonium acetate (2 N) were then added, resulting in a pH ranging from 5.1 to 5.2. The solution was stirred vigorously and set aside overnight, then filtered through a Whatman No. 41 filter paper and washed with cold water. After drying, the precipitate was ashed with the paper in a porcelain crucible at 450° C. in an electric muffle.

Spectrographic Procedure

The ash obtained from the 8-hydroxyquinoline concentration was transferred to a small glass vial and dissolved in a minimum of hydrocloric acid (1:1). The solution was then evaporated to dryness and taken up in two ml of buffer solution containing the internal standard. The solution was thoroughly mixed, and, with a serological pipet, a 0.10 ml aliquot of the unknown or standard solution was placed on each of the two prepared electrodes. The electrodes with the solutions were placed in an air oven at 100° C. for several hours, preferably overnight. A duplicate set of electrodes was needed because of the double exposure used. A series of standards was run on each plate.

The samples were arced using a d.c. potential of 120 volts and a current of eight amperes for a period of 30 seconds with the sector 5/16 open. The slit was set at 45 mμ.

After exposure the plates were developed four and one half minutes at 68° C. in Eastman X-ray developer diluted two to one. They were then fixed, dried, and the line intensities determined with the use of an A.R.L.-Dietert densitometer. Eastman 33 plates were used throughout the procedure.

The results were expressed in parts per million of zinc, on the dry weight basis.

Precision of Analysis Using the Zinc Line at 3345.0 Angstroms

The precision of analysis using this line was determined for two samples differing widely in concentration. Table 5 presents data used to calculate the precision of analysis of a sample from Norton containing 23.9 ppm of zinc. The probable error was found to be 2.42 percent, which is lower than the probable error in the analysis of a sample from Burlington containing 52.1 ppm. Table 6 presents the data obtained from the Burlington sample in which the probable error was 3.36 percent.

Since the precision of this method is considerably better

Table 5. Analyses of alfalfa from Norton to show precision of spectrographic method using the line at 3345.0 Å.

Cd	Zn	Cd/Zn	ppm	D	D^2
9.0	73.8	.122	19.5	4.4	19.36
9.5	76.0	.125	20.5	3.4	11.56
10.0	69.3	.145	27.0	3.1	9.61
8.8	59.8	.147	27.5	3.6	12.96
9.5	71.0	.134	25.0	1.1	1.21
9.3	66.8	.139	25.5	1.6	2.56
9.8	70.3	.139	25.5	1.6	2.56
10.8	75.0	.143	26.2	2.3	5.29
11.8	75.0	.156	28.3	4.4	19.36
10.3	82.5	.124	20.5	3.4	11.56
9.8	75.3	.129	22.2	1.7	2.89
9.0	72.5	.124	20.5	3.4	11.56
8.0	64.0	.125	20.5	3.4	11.56
10.0	70.3	.142	26.0	2.1	4.41
Total			334.7		126.45
Average			23.9		

S = standard deviation = $\sqrt{\frac{\Sigma D^2}{n-1}}$ = $\sqrt{\frac{126.45}{13}}$ = 9.73 = 3.12

Standard error = S_x = $\frac{S}{\sqrt{n}}$ = $\frac{3.12}{\sqrt{14}}$ = $\frac{3.12}{3.74}$ = .832

Probable error = $S_x \cdot \frac{\sqrt{2}}{2}$ = (.832)(0.7) = .582

Percent standard error = $\frac{.832}{23.9}$ = 3.46 percent

Percent probable error = $\frac{.582}{23.9}$ = 2.42 percent

Table 6. Analyses of alfalfa from Burlington to show precision of spectrographic method using the line at 3345.0 Å.

Cd	Zn	Cd/Zn	ppm	D	D^2
11.0	51.6	.216	46.3	5.8	33.64
10.3	43.5	.230	51.3	0.8	0.64
12.2	49.3	.250	57.5	5.4	29.16
11.2	45.5	.246	57.0	4.9	24.01
12.4	46.5	.267	61.3	9.2	84.64
11.5	55.5	.210	45.0	7.1	50.41
10.0	46.5	.215	46.0	6.1	37.21
Total			364.4		259.71
Average			52.1		

Standard deviation = $\sqrt{\frac{\Sigma D^2}{n-1}}$ = $\sqrt{\frac{259.71}{6}}$ = 6.56

Standard error = $S_{\bar{x}}$ = $\frac{S}{\sqrt{n}}$ = $\frac{6.56}{\sqrt{7}}$ = 2.5

Probable error = $S_{\bar{x}} \cdot \frac{\sqrt{2}}{2}$ = (2.5)(0.7) = 1.75

Percent standard error = $\frac{2.25}{52.1}$ = 4.8 percent

Percent probable error = $\frac{1.75}{52.1}$ = 3.36 percent than that obtained using the 2138.6 Angstrom line without concentration, it was decided to use the preliminary chemical concentration of the zinc followed by analysis using the line located at 3345.0 Angstroms.

COMPARISON OF SPECTROGRAPHIC ANALYSIS USING THE 3345.0 ANGSTROM LINE AND DITHIZONE CHEMICAL METHOD

Before spectrographic procedure can be accepted with any degree of confidence, it must undergo a favorable comparison with a well-known chemical procedure. The method used for comparison was identical to the widely used dithizone colorimetric procedure of Cowling and Miller (9). A two and one half g sample of dry plant material was ashed and the zinc extracted in the same manner as for the 8-hydroxyquinoline concentration. The filtrate from the hydrochloric acid extraction was collected in a 100 ml volumetric flask, one drop of methyl red added and neutralized with normal ammonium hydroxide. Four ml of normal hydrochloric acid were added and the contents made to volume. A 20 ml aliquot of the sample was pipeted into a 125 ml separatory funnel. The zinc and other elements, such as copper, lead, and cobalt form a complex with dithizone in carbon tetrachloride and are extracted at pH 8.5 to 9.0. The zinc was separated from the copper and excess reagent by extraction into 0.02 N hydrochloric acid. The pH was adjusted to between 8.5 and 9.0 by means of an ammonia-ammonium citrate buffer and the zinc again extracted with dithizone in the presence of carbamate to prevent interference by other elements. A five ml aliquot of the extract was placed in a photoelectric colorimeter tube (an Evelyn Photoelectric Colorimeter manufactured by the Rubicon Instrument Company of Philadelphia) and diluted with 20 ml of pure carbon tetrachloride and thoroughly mixed. The percent transmission was determined using a 520 mμ filter, and the concentration of zinc was determined by comparison with the curve shown in Fig. 4.

A series of nine alfalfa samples were analyzed by this method and compared with spectrographic analysis using the zinc line at 3345.0 Angstroms. The results are tabulated in Table 7. Examination of the data shows a maximum deviation of 21.2 percent, the average deviation being 5.5 ppm. The spectrographic analysis results average approximately 13.6 percent below the colorimetric procedure.

RESULTS OF THE ALFALFA SURVEY

The samples used for the survey were obtained from several localities within the state. These areas were chosen in such a manner as to represent several of the different soil types found in Kansas. All samples were secured at approximately the same stage of growth, that of about one-tenth bloom. This method of sampling would tend to eliminate differences due to variations in zinc content caused by collecting samples of different physiological stages of growth. Fields were sampled by obtaining small amounts of

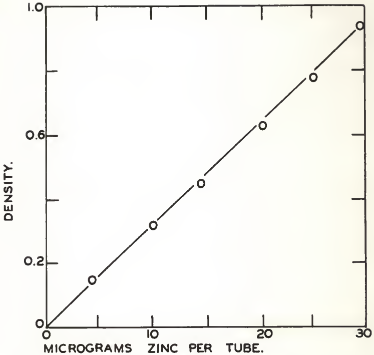

FIG. 4. COLORIMETRIC WORKING CURVE.

Table 7. Comparison of spectrographic and dithizone methods of analysis of alfalfa for zinc using the line at 3345.0 Å.

Sample	Dithizone ppm	Spectrographic ppm	Deviation	Percent deviation
Norton	33.3	26.3	-7.0	-21.2
Larned	35.1	28.8	-6.3	-17.9
Hiawatha	35.2	28.8	-6.4	-18.3
Kingman	35.4	28.7	-6.7	-18.9
Meade	38.7	34.0	-4.7	-12.3
Manhattan	39.2	36.8	-2.4	- 6.1
Wakefield	41.7	38.3	-2.4	- 6.0
Burlington	62.0	52.5	-10.5	-16.9
Columbus	68.0	65.0	-3.0	- 4.5
Average			-5.5	-13.6

alfalfa from several places within the field and then dividing the sample in the usual manner.

A series of samples of alfalfa being grown on fertilizer plots in two different places in the state were also obtained. These were secured from plots near Thayer and Columbus in order to show the effects of different soil treatments on the zinc content of the plant material.

An outline map of Kansas is shown in Fig. 5, including the locations from which samples were secured. The general classification of the soils in these areas, as well as in the entire state, can be readily obtained by reference to a map of Kansas prepared by the Soil Conservation Service (13).

The data in Table 8 represent average analysis of alfalfa grown on untreated plots in the locations given. Four of the samples in the eastern region of the state show the highest zinc content exhibited in the survey. The samples in the east central area show slightly lower zinc content, averaging approximately 35 ppm. In general, the zinc content decreased across the state, being higher in the east and lower in the west. It is of interest to note that the four extreme eastern samples which show the highest zinc content have the lowest ash content. The ash content, which is indicative of the total mineral content, is lower in the eastern regions. This trend may be partially expected, however, on the basis of rainfall data and the length of time the land has been farmed. Zinc has been shown by Stokes and Cummings (40) to be held in the soil largely in a non-water soluble form. The

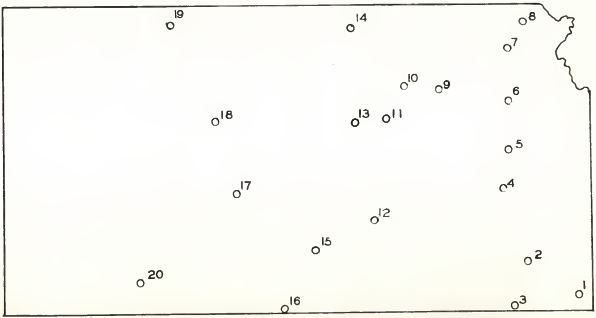

FIG.5. KANSAS MAP SHOWING WHERE ALFALFA SAMPLES WERE OBTAINED.

Table 8. Zinc and ash content* of alfalfa taken from untreated plots** in Kansas.

Locality		Ash percent	Zinc ppm	Locality		Ash percent	Zinc ppm
Columbus	(1)	7.35	65.0	Abilene	(11)	10.94	33.9
Thayer	(2)	7.79	28.7	Sedgwick	(12)	10.87	25.1
Burlington	(4)	7.93	52.5	Salina	(13)	10.54	33.7
Lyndon	(5)	9.44	48.4	Belleville	(14)	8.97	26.3
Topeka	(6)	7.87	88.8	Kingman	(15)	9.92	28.7
Netawaka	(7)	9.44	29.4	Kiowa	(16)	10.00	23.9
Hiawatha	(8)	10.26	28.8	Larned	(17)	11.25	28.8
Manhattan	(9)	10.90	36.8	Hays	(18)	12.67	31.2
Wakefield	(10)	11.45	38.3	Norton	(19)	11.43	26.3
				Meade	(20)	12.18	34.0

* Figures are reported on a dry weight basis.

** Number following locality corresponds to those on Fig. 5.

average rainfall in the Columbus area has averaged 41.8 inches annually, according to records of the weather bureau, while near Salina the average has been 27.3 inches. This decreases correspondingly further west.

The data presented in Table 9 indicate the effect of different fertilizer treatments on the quantity of zinc found in plant material. The plots on which the fertilizer treatments were carried out are located comparatively close together in the south eastern corner of the state. The untreated sample from Thayer was one of the exceptions to the comparatively high zinc content of the samples in this region.

The data in Table 9 indicated that application of fertilizer to samples grown at Columbus in nearly every case caused a decrease in the ash content. The zinc content also was decreased in all except two cases. Application of lime, manure, and superphosphate in one of the two cases resulted in a slight increase of zinc. Each of the fertilizers applied alone, however, caused a decrease in zinc. Lime and superphosphate applied together in one case out of four caused an increase, the remainder showing lower zinc content.

Results of an opposite nature were obtained from application of the same fertilizers to plots at Thayer. In all except one case the ash content increased, and only two samples showed a decrease in zinc. Either lime or manure applied separately caused large increases in zinc, while the two together show a comparatively small increase. Lime in conjunction with manure and rock phosphate showed an increase

Table 9. Zinc content in alfalfa taken from treated plots at Thayer and Columbus

	Thayer							Columbus						
	Amount					Analysis		Amount					Analysis	
Treatment*	L ton	M ton	S lb.	R ton	K lb.	Zinc ppm	Ash percent	L ton	M ton	S lb.	R ton	K lb.	Zinc ppm	Ash percent
None						28.7	7.79						65.0	7.35
M		8				59.4	7.60							
L	2					68.4	8.36	1.5					47.8	8.01
L. M.	2	8				30.6	8.57	1.5	8				36.2	7.58
L. M. S.	2	8	150			23.5	9.07	1.5	8	120			36.4	7.22
								1.5	8	120			69.5	6.88
L. M. R.	2	8		1/2		39.4	8.73							
L. S.	2		150			30.4	8.54	1.5		120			67.5	6.75
						26.6	8.73	1.5		120			32.5	6.02
								1.5		120			27.4	6.62
								1.5		120			33.9	6.53
L. R.								1.5			1/2		49.2	6.91
L. 2xS appl. Bien.	2		300			50.0	8.77							
L. S. K.	2		150		20	34.8	8.14	1.5		120		16	57.2	6.93

*M is manure; L is ground limestone; S is 20% superphosphate; R is ground rock phosphate and K is muriate of potash (50%). The figures relate to quantities per acre and are given in pounds and tons. 2xS appl. bien. means a double application of superphosphate 20% applied biennially.

of approximately 10 ppm zinc. Lime and double applications of 20 percent superphosphate applied biannially also show a considerable increase in zinc.

Lott (21) has shown the availability of zinc in soils dependent on pH. At a pH below 6 zinc seems more readily available and above pH 6 much larger concentrations of zinc do not show toxic effects. A partial explanation of the results shown in Table 9 may lie in different soil types with respect to pH and total zinc available to the plant. It is also possible that various fertilizers cause fixation of zinc in such a manner that it is no longer available to the plant.

In order to show the effect of the stage of growth on the zinc content of alfalfa, samples were taken at Manhattan every seven days from the time the alfalfa first showed spring growth until the time of cutting. The data obtained, which are presented in Table 10, indicated that apparently the zinc content decreases slightly during growth. As the plant matures the total mineral content shows an irregular increase, although the zinc does not seem to decrease in the same ratio.

Table 10. Zinc content* of Manhattan grown alfalfa at seven day intervals up to time of cutting.

Time days	Height inches	Ash percent	Zinc ppm
7	5	9.73	47.5
14	10	10.89	26.3
21	14	10.52	41.0
28	17	10.86	38.0
35	21	10.15	38.7
42	24	9.38	34.3

* All analyses reported on dry weight basis.

CONCLUSIONS

A spectrographic procedure of good precision for the determination of zinc in plant ash has been developed. The procedure made use of 8-hydroxyquinoline to concentrate the zinc and lithium chloride as a buffer to suppress variations due to differences in composition and to stabilize the d.c. arc. Cadmium was used as the internal standard.

The precision of this method showed a probable error of less than three percent while the analysis of alfalfa using the zinc line at 2138.6 Angstroms showed a probable error of greater than 15 percent. The spectrographic method using the zinc line at 3345.0 Angstroms gave values which compared satisfactorily with the dithizone colorimetric procedure, the average deviation being 13.6 percent below the chemically determined value.

This method was applied successfully to alfalfa plant material, and a survey of the zinc content of alfalfa taken from several areas in Kansas was conducted. The data do not show any area exceptionally low in zinc and since the zinc requirement of alfalfa has not been established no conclusions regarding a possible zinc deficiency in Kansas grown alfalfa could be drawn. Since the effect of fertilization varied with the two experimental plots, there was apparently no correlation between the zinc uptake of the plant and the type of fertilizer. Lime, manure, and superphosphate separately and in combination appear to cause increased uptake of zinc in some cases. Analyses of alfalfa at various stages of maturity showed a gradual decline in the zinc as correlated with an irregular increase of total mineral content.

ACKNOWLEDGMENTS

The writer wishes to thank Dr. W. G. Schrenk for his aid in the selection of this problem, his liberal donation of time and his helpful suggestions and supervision during this investigation. Thanks are also due to the Department of Agronomy of Kansas State College and to C. O. Grandfield and Harold Hackerott of the United States Department of Agriculture for collecting the samples that were used.

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